Effects of Supplementation of Energy or Ruminally Undegraded Protein to Lactating Cows Fed Alfalfa Hay or Silage[1]

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ABSTRACT

Alfalfa was harvested as silage or hay and fed in two 12-wk trials with a 4 × 4 Latin square design that used 12 (trial 1) or 24 (trial 2) multiparous lactating cows (4 ruminally cannulated cows per trial). Diets contained (dry matter basis) 75 or 50% alfalfa plus 24 or 40% high moisture corn (trial 1) or 50% alfalfa, 44 or 41% high moisture corn, with (3%) or without fish meal (trial 2). Experiments were conducted to evaluate the responses of cows fed alfalfa hay or alfalfa silage diets to an increase in protein supply from microbial protein synthesis (trial 1) or from the supplementation of ruminally undegraded protein (RUP) (trial 2). In trial 1, the increase in high moisture corn in the diet increased both milk protein and microbial crude protein yields (estimated from the excretion of purine derivatives) to a greater extent for the cows fed the alfalfa silage diets (170 and 337 g/d, respectively) than for the cows fed the alfalfa hay diets (100 and 100 g/d, respectively). In trial 2, RUP supplementation (as fish meal) increased milk protein yield 100 g/d for cows fed alfalfa silage diets and 20 g/d for cows fed alfalfa hay diets. These results indicated that protein status was poorer and, thus, more responsive to absorbable protein from microbial protein (trial 1) or RUP (trial 2) for cows that consumed alfalfa conserved as silage versus those that consumed alfalfa conserved as hay.
(**Key words**: alfalfa, purines, purine derivatives, microbial protein synthesis)

Abbreviation key: **AH** = alfalfa hay, **AS** = alfalfa silage, **FM** = fish meal, **HMC** = high moisture corn, **MCP** = microbial CP, **PD** = purine derivatives.

INTRODUCTION

Because of its high yield, high protein content, and relatively low fiber content, alfalfa is the principal forage in many dairy rations in the US. Ease of mechanization and reduced susceptibility to weather damage have made conservation of alfalfa as silage, rather than as hay, increasingly common. Crude protein is typically the most expensive nutrient in dairy rations, and alfalfa often supplies more than 50% of the dietary CP; therefore, utilization of CP in alfalfa silage (**AS**) and alfalfa hay (**AH**) is of great importance. Substantial increases in secretion of milk and milk protein as a result of the supplementation of RUP (4, 13) or abomasal casein infusion (12) in cows fed AS diets indicated that intestinal protein supply was limiting. Moreover, response of milk protein secretion to RUP was greater for cows fed AS diets than for cows fed AH diets (4). The extensive conversion of protein to NPN that occurs during silage fermentation (21) results in excessive production of NH_3 in the rumen (11), which suggests that conservation of alfalfa as silage may reduce ruminal protein escape, synthesis of ruminal microbial CP (**MCP**), or both, relative to conservation of alfalfa as hay.

The inclusion of ground high moisture corn (**HMC**) in the diets of dairy cows stimulated the uptake of NH_3 in vitro and also stimulated milk protein secretion (1) relative to the inclusion of unground HMC. These results suggested that enhancing the availability of ruminal fermentable energy might be an effective strategy to increase microbial capture of RDP from AS. Supplementation of AS with high moisture ear corn increased milk protein secretion by 260 g/d (8), presumably through enhanced ruminal MCP synthesis.

Based on the correlation between purine concentrations in digesta and excretion of purine derivatives

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[1]Mention of any trademark or proprietary product in this paper does not constitute a guarantee or warranty of the product by the USDA or the Agricultural Research Service and does not imply its approval to the exclusion of other products that also may be suitable.
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TABLE 1. Composition of diets.[1]

Item	Trial 1				Trial 2			
	AH Plus 24% HMC	AS Plus 24% HMC	AH Plus 40% HMC	AS Plus 40% HMC	AH	AS	AH Plus 3% FM	AS Plus 3% FM
	(% of DM)							
AH	75.0	...	55.0	...	50.0	...	50.0	...
AS	...	75.0	...	55.0	...	50.0	...	50.0
Ground HMC	24.0	24.0	40.0	40.0	44.1	44.1	41.1	41.1
Soybean meal	...	...	3.5	3.5	4.0	4.0	4.0	4.0
FM	...	...	...	...	...	...	3.0	3.0
Sodium bicarbonate	...	...	0.5	0.5	0.5	0.5	0.5	0.5
Sodium phosphate	0.5	0.5	...	...	...	...	...	...
Dicalcium phosphate	...	...	0.5	0.5	0.7	0.7	0.7	0.7
Trace-mineralized salt[2]	0.3	0.3	0.3	0.3	0.5	0.5	0.5	0.5
Vitamin premix[3]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Dynamate®[4]	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Chemical composition								
CP	17.0	17.3	16.2	16.4	15.3	16.5	17.2	18.4
NDF	35.6	33.0	30.4	28.5	28.9	26.2	28.6	25.9
ADF	25.0	23.8	19.9	19.1	18.8	17.0	18.7	16.8
NE_L,[5] Mcal/kg of DM	1.55	1.61	1.67	1.70	1.70	1.76	1.71	1.76

[1]AH = Alfalfa hay, AS = alfalfa silage, HMC = high moisture corn, and FM = fish meal.

[2]Provided 16 mg of Mn, 16 mg of Zn, 10 mg of Fe, 4 mg of Cu, 24 mg of I, 0.2 mg of Se, and 0.06 mg of Co/kg of DM.

[3]Provided 3880 IU of vitamin A, 730 IU of vitamin D, and 0.73 IU of vitamin E/kg of DM.

[4]Magnesium and potassium sulfate (Marshall Minerals, Marshall, TX).

[5]Computed using NE_L contents estimated from forage NDF (20) and from NRC (22) tables for other ingredients.

(**PD**) in urine that was reported by Topps and Elliott (28) and on the potential for estimating MCP flow from PD excretion, recent work has explored the quantitative relationship between purine flow in digesta and excretion of PD in sheep (9) and steers (30). Vagnoni et al. (29) recently described the relationship between the excretion of PD and purine flow in the intestines of dairy cows. Trial 1 was conducted to compare the response of milk protein secretion from cows fed AH and AS diets supplemented with HMC and to determine whether responses corresponded with MCP supply as estimated by PD excretion. Trial 2 was conducted to replicate previously observed responses in milk protein secretion from cows fed AH and AS diets supplemented with fish meal (**FM**) (4) but at forage concentrations reduced to 50% of dietary DM to represent more nearly practical dairy diets.

MATERIALS AND METHODS

Trial 1

Cows and feeding. The AS was chopped to a theoretical length of 1.0 cm and ensiled in a concrete bunker silo at 41% DM. The AH was wilted to approximately 85% DM, conserved as small rectangular bales, and stored under shelter. Neither hay nor silage was rained on. Twelve multiparous Holstein cows at a mean (±SD) of 603 ± 38 kg, at 128 ± 96 DIM, and yielding 36 ± 5 kg/d of milk were assigned to three replicated 4 × 4 Latin squares with 21-d periods. Days 1 to 14 of each period served as the adaptation period, and all samples and data were collected from d 15 to 21. Square 1 consisted of ruminally cannulated cows; the remaining 8 cows were assigned to squares 2 and 3 according to DIM. Cows within squares were randomly assigned to treatment sequences. All cows were administered bST (500 mg/d of Posilac®; Monsanto, St. Louis, MO) before the initiation of the trial and were injected at 14-d intervals throughout. Diets (Table 1) were based on AH or AS and contained (DM basis) 24 or 40% HMC. The HMC was ground through a 1-cm screen using a hammer mill and then mixed in the diet.

The TMR were fed for ad libitum intake once daily at 1000 h by adjusting the daily feed offered to yield about 5% orts. Weekly composites of AS, chopped AH, HMC, TMR, and orts were prepared from daily samples of about 0.5 kg stored at –20°C. A weekly sample of the soybean meal was also taken. The AS and HMC contents of the diets (as-fed basis) were adjusted as needed based on DM, which was determined weekly at 60°C (48 h). Cows had free access to water throughout the trial. Milk yield was recorded daily at both the a.m. and p.m. milkings. One cow became sick

during period 3, as was evidenced by a sharp decline in DMI and milk yield. This cow was treated once with 20 ml of penicillin (300,000 U/ml) intramuscularly, and all data for this cow from that period were deleted. The cow recovered during the adaptation part of period 4; DMI and milk yield data returned to normal, and, thus, all data from this cow for period 4 were used.

Sampling. Total urine collections were made using indwelling Foley catheters (24 French, 75-ml balloons; C. R. Bard, Murray Hill, NJ), which were inserted at 1300 h on d 18 of each experimental period; urine output was measured every 12 h for 3 d. Fresh containers with 1 L of 20% (vol/vol) H_2SO_4 were attached to catheters at 0400 and 1600 h to obtain a.m. and p.m. samples (final pH <3). Just prior to milking, catheters were clamped shut, and cows were led to the milking parlor, milked, and immediately returned to their stalls; fresh containers were then attached. Catheters remained clamped for approximately 45 min at each milking. After the weight of the acidified urine was recorded, its specific gravity was determined using a hydrometer (number 11-603-7A; Fisher Scientific, St. Louis, MO); 10-ml aliquots were diluted to 100 ml with tap water and stored at –20°C. Urinary volume was computed as the quotient of the weight of urine excreted divided by its specific gravity. Milk samples also were obtained at the a.m. and p.m. milkings for the last 3 d of each experimental period.

Samples of whole ruminal contents were obtained from cannulated cows at 0, 2, 4, 6, 8, 10, and 12 h postfeeding on the last day of each experimental period. Samples (consisting of 200 ml of subsamples from five locations in the rumen) were strained through two layers of cheesecloth, and the pH of the resulting strained ruminal fluid was determined using a combination electrode (Corning model 360i pH meter; Corning Inc., Corning, NY). Subsamples of ruminal fluid were then preserved with the addition of 1 ml of 50% (vol/vol) H_2SO_4/50 ml of ruminal fluid and stored at –20°C. Additional ruminal samples (1 L) were obtained at each sampling time (0, 4, 8, and 12 h) and preserved with formalin (25 ml of formalin/L of ruminal contents). These digesta samples were stored at 4°C for approximately 12 to 24 h before being squeezed through two layers of cheesecloth and then were washed three times with a total of 3 L of McDougall's buffer. Ruminal fluid plus buffer wash was centrifuged (550 × *g* at 4°C for 10 min), the supernatants were decanted and centrifuged (15,000 × *g* at 4°C for 20 min), and the resulting bacterial pellets were dried at 60°C for 48 h.

Blood samples were taken 4 h after feeding on the last day of each period from the coccygeal artery or vein. Samples were heparinized, held at 4°C overnight until plasma was prepared, deproteinized using four volumes of plasma to one volume of 15% (wt/vol) 5-sulfosalicylic acid, and stored at –20°C.

Laboratory analyses. Weekly composites of TMR and feed ingredients were analyzed for total N (Carlo Erba NA 1500 N analyzer; Carlo Erba Instruments, Milan, Italy), DM and OM (2), and NDF and ADF (24). Silage extracts were prepared from silage samples in distilled water (21) and analyzed for NPN after precipitation of the protein with TCA [20 ml of silage extract plus 5 ml of 25% (wt/vol) TCA]. Milk samples were split. One subsample was analyzed for fat, protein, lactose, and SNF by infrared analysis (Wisconsin DHI Cooperative, Madison), and the other was diluted 1:1 (vol/vol) with 25% (wt/vol) TCA and centrifuged (15,000 × *g* at 4°C for 20 min); the supernatant was stored at –20°C. Urine and milk samples that were treated with TCA were analyzed for uric acid and allantoin (14). Plasma was analyzed for allantoin (14), and urine was analyzed for creatinine (23). Acidified ruminal fluid was thawed, centrifuged (30,000 × *g* at 4°C for 15 min), and analyzed

TABLE 2. Composition of alfalfa forages fed during trials 1 and 2.[1]

	Trial 1		Trial 2	
Component	AS	AH	AS	AH
DM, %	41.4	85.0	44.5	84.8
NDF, % of DM	38.6	42.0	38.2	43.4
ADF, % of DM	29.9	31.4	28.9	32.6
CP, % of DM	20.1	19.8	21.2	18.8
NPN,[2] % of Total N	56.9	ND[3]	42.9	ND
NE_L,[4] Mcal/kg of DM	1.50	1.42	1.50	1.39

[1]AS = Alfalfa silage; AH = alfalfa hay.

[2]Proportion of total N soluble in 5% (wt/vol) TCA (21).

[3]Not determined.

[4]Values for NE_L for alfalfa forages computed from NDF using the equation of Mertens (20).

for NH_3 and total free AA (5). Areas under time-course curves for ruminal pH, NH_3, and total free AA were calculated using the trapezoidal rule, and mean concentrations were computed by dividing areas by the number of hours (12 h). To reduce the number of VFA determinations, cow by period composites of ruminal fluid were prepared and analyzed (31). Total VFA concentrations were computed as the sum of acetate, propionate, and butyrate, which represented greater than 90% of all VFA (17). Dried bacterial samples were ground in a coffee grinder and analyzed for total N (Carlo Erba NA 1500 N analyzer) and for purines by HPLC (29).

Microbial CP flow was estimated using the following equation (29): MCP (grams per day) = (grams of MCP/millimoles of purine) × [(millimoles of PD excreted per day – 103)/0.856].

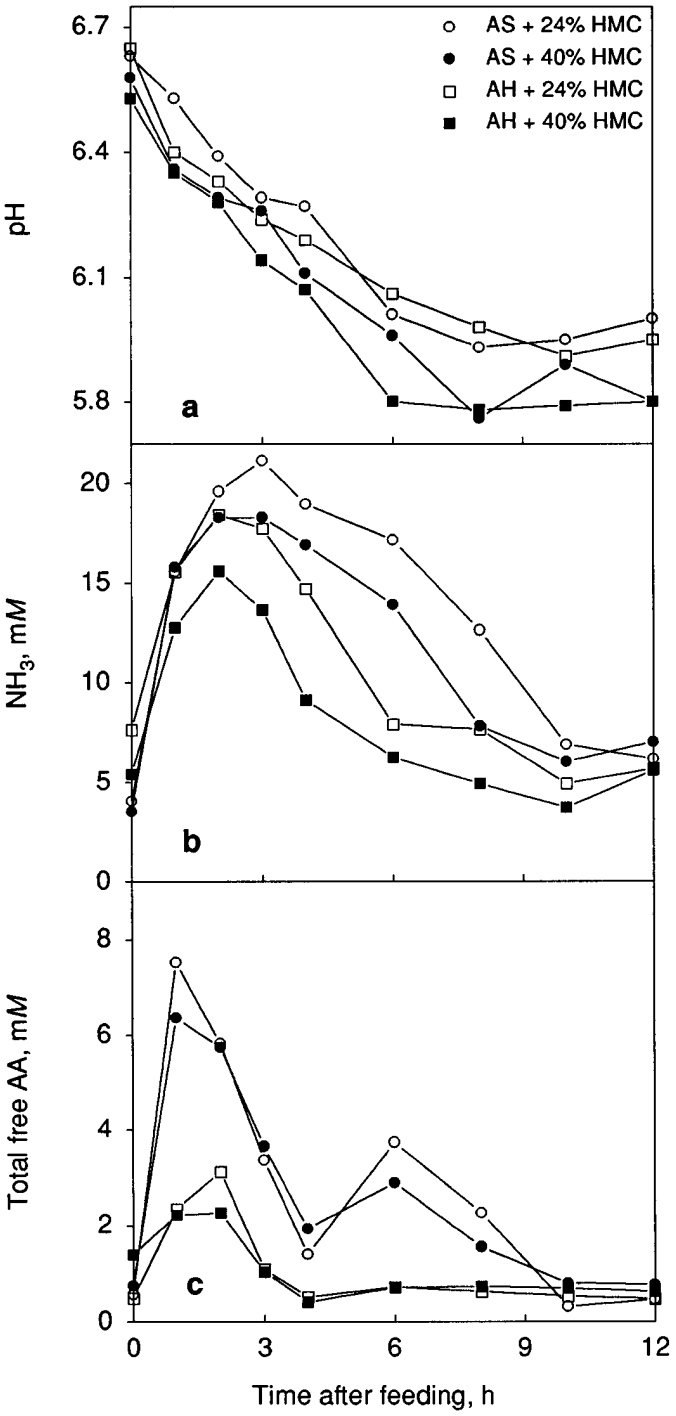

Figure 1. Hourly means from trial 1 for ruminal pH (pooled SE = 0.04) (a), ruminal NH_3 (pooled SE = 0.7) (b), and ruminal total free AA (pooled SE = 0.3) (c). AS = Alfalfa silage, AH = alfalfa hay, and HMC = high moisture corn.

Trial 2

The procedures used in trial 2 were similar to those in trial 1. Twenty-four multiparous Holstein cows used in this trial, 4 of which were ruminally cannulated, cows weighed ($\bar{X} \pm SD$) 594 ± 48 kg, were 46 ± 11 DIM, and yielded 42 ± 5 kg/d of milk; blocking and assignment to the 4 × 4 Latin squares were as in trial 1, and periods were also 21 d long. Cows were injected with bST as in trial 1, but at 21-d intervals. Diets (Table 1) consisted of 50% alfalfa and 50% concentrate (largely HMC) on a DM basis; diets were formulated to contain 28% NDF. To examine the response to RUP supplementation, ruminant-grade FM (Sea Lac®; Zapata Protein, Inc., Hammond, LA) was either not added or was added at 3% of dietary DM at the expense of HMC. Feeding, feed sampling, and analyses were as in trial 1. Because the responses of milk yield to the infusion of postruminal protein occur within 24 h (6), 1 wk was considered adequate for adaptation to RUP, and data were collected over the last 2 wk of each period.

Milk yield was recorded daily at both the a.m. and p.m. milkings, and milk was sampled at one p.m. milking and one a.m. milking midway through wk 2 and 3 of each period and analyzed as in trial 1, except that PD were not determined. Ruminal fluid was collected from cannulated cows at 0, 1, 2, 3, 4, and 6 h after feeding on the last day of each period and analyzed as in trial 1.

Statistical Analyses

Data were analyzed as a 4 × 4 Latin square, replicated three (trial 1) or six (trial 2) times for DMI, milk yield and composition, blood plasma data, excretion of PD, and MCP flow, and replicated once for ruminal data using the general linear models procedure of SAS (25). The model for the replicated Latin square included square, cow within square, period, diet, the interaction of period and square, and the

interaction of diet and square. For all responses, the effects of square ($P \geq 0.276$) and interactions ($P \geq 0.058$) were not significant; these data were pooled with the residual. The model for the ruminal Latin square consisted of cow, period, and diet. For the MCP to purine ratio, time was added to the model as a repeated factor, and the nature of the time effect was evaluated with linear, quadratic, and cubic orthogonal polynomials. All dietary effects were evaluated using three orthogonal contrasts: 1) forage source (AH vs. AS), 2) dietary HMC (trial 1) or FM (trial 2), and 3) the interaction of forage source and HMC (trial 1) or forage source and FM (trial 2).

RESULTS AND DISCUSSION

Trial 1

Although CP was similar, concentrations of NDF and ADF were, respectively, 3.4 and 1.5 percentage units higher in AH than in AS (Table 2). Consequently, the fiber content was higher, and the calculated NE_L content (20) was lower, in AH diets than in AS diets (Table 1). The high NPN content (56.9%) of AS (Table 2) was in accordance with typical observations in excess of 50% (4). Although not determined in the present experiment, the NPN content of AH is usually about 10% of total N (4).

Within 4 h after feeding, the ruminal pH of cows fed all diets was <6.3 (Figure 1a), which was the pH identified as critical for maintaining ruminal fiber digestion (16, 27); pH remained <6.3 throughout the remaining 8 h during which measurements were made. Mean pH (Table 3) was reduced ($P = 0.046$) 0.12 units by increasing dietary HMC, but forage source had no effect. Concentrations of ruminal NH_3 were greater ($P = 0.006$) for cows fed AS diets than for cows fed AH diets and were decreased ($P = 0.033$) in response to HMC (Table 3; Figure 1b). Concentrations of NH_3 for all diets remained above the 3.6 m*M* value suggested (26) as the minimum value that was necessary to maintain ruminal bacterial growth; insufficient ruminal NH_3 would rarely be a concern for diets based on alfalfa. The peak in ruminal total free AA concentrations observed at 1 h postfeeding (Figure 1c) for cows fed AS diets was consistent with previous findings (11) and reflected the extensive ruminal degradation of silage CP as well as the large amount of N in AS that was present as peptides and free AA. Mean concentrations of total free AA (Table 3) were higher ($P = 0.005$) for cows fed AS diets than for cows fed AH diets (2.54 vs. 0.97 m*M*) but were not affected by amount of HMC or the interaction of forage and HMC.

Total VFA concentrations ($\overline{X}$ = 143 m*M*; Table 3) were not affected by forage source ($P = 0.540$) or,

TABLE 3. Effect of diet on ruminal pH and metabolite concentrations.[1]

Ruminal fluid	AH Plus 24% HMC	AS Plus 24% HMC	AH Plus 40% HMC	AS Plus 40% HMC	SE	$P > F^2$ Forage	HMC	Forage × HMC
Trial 1								
pH	6.12	6.14	5.98	6.04	0.04	0.402	0.046	0.685
NH_3, m*M*	10.3	13.8	8.5	11.5	0.7	0.006	0.033	0.732
Total free AA, m*M*	0.97	2.68	0.97	2.41	0.30	0.005	0.701	0.711
Total VFA, m*M*	137	147	145	143	6	0.540	0.779	0.318
Acetate (A), mol/100 mol of VFA	70.8	67.0	67.1	65.7	0.7	0.011	0.014	0.142
Propionate (P), mol/100 mol of VFA	18.7	21.9	22.1	22.3	0.7	0.068	0.048	0.094
Butyrate, mol/100 mol of VFA	10.5	11.1	10.7	12.0	0.2	0.011	0.061	0.174
A:P	3.80	3.07	3.09	2.96	0.14	0.030	0.035	0.092
	AH	AS	AH Plus 3% FM	AS Plus 3% FM		Forage	FM	Forage × FM
Trial 2								
pH	6.05	6.16	6.18	6.21	0.10	0.515	0.436	0.733
NH_3, m*M*	12.2	13.8	12.6	15.4	0.8	0.039	0.302	0.521
Total free AA, m*M*	1.36	2.26	1.19	1.90	0.30	0.034	0.402	0.768
Total VFA, m*M*	168	165	156	157	6	0.874	0.166	0.777
Acetate (A), mol/100 mol of VFA	66.5	64.5	69.4	66.6	1.6	0.204	0.181	0.807
Propionate (P), mol/100 mol of VFA	22.7	23.0	20.2	21.4	1.7	0.662	0.280	0.797
Butyrate, mol/100 mol	10.9	12.5	10.4	12.0	0.2	<0.001	0.089	0.889
A:P	3.09	2.91	3.47	3.16	0.30	0.444	0.328	0.836

[1]AH = Alfalfa hay, AS = alfalfa silage, HMC = high moisture corn, and FM = fish meal.

[2]Probability of a significant contrast effect.

surprisingly, by amount of HMC ($P = 0.779$). Concentrations of VFA represent a balance between production and disappearance, and important differences in production rate may not be apparent from VFA concentrations (18). The molar percentage of acetate was lower, and the molar percentage of propionate was higher ($P \leq 0.068$), for cows fed AS than for cows fed AH diets. The effects on acetate and propionate were similar for increased dietary HMC. Consequently, the acetate to propionate ratio was decreased ($P \leq 0.035$) because of both dietary factors. Although milk fat percentage often reflects the acetate to propionate ratio, there were no dietary effects on milk fat content ($P \geq 0.314$; Table 4). The molar percentage of butyrate was lower ($P = 0.011$) for cows fed the AH diets than for cows fed the AS diets. The molar percentage of butyrate was higher ($P = 0.061$) because of the addition of HMC.

Daily DMI (Table 4) was 0.8 kg higher ($P = 0.044$) for cows fed AH diets than for cows fed AS diets; daily DMI was increased 1.5 kg ($P < 0.001$) in response to HMC addition. There was no interaction between forage source and HMC. Milk yield was not affected by forage source, but was increased 2.8 kg/d ($P < 0.001$) in response to HMC. No interaction was detected ($P = 0.262$) between forage source and HMC despite numerically greater milk yield in response to HMC for cows fed the AS diets (3.6 kg/d) than for cows fed the AH diets (2.0 kg/d). Milk protein content was increased ($P = 0.005$) by AH versus AS, and milk content of protein, lactose, and SNF all increased ($P \leq 0.007$) in response to HMC. No interactions were detected for the concentrations of any milk components. Yields of milk components were not affected by forage source, but all increased ($P < 0.001$) in response to HMC. Moreover, yields of all components were affected ($P \leq 0.100$) by an interaction between forage source and HMC; HMC increased yields by 5 to 10% and 13 to 19% on AH and AS diets, respectively. Efficiency of milk yield was not affected by diet.

Excretion of urinary allantoin, uric acid, and total PD (Table 5) was higher ($P \leq 0.029$) for cows fed AH than for cows fed AS diets and also increased ($P <$

TABLE 4. Effect of diet on DMI, BW gain, and yield of milk and milk components.[1]

	AH Plus 24% HMC	AS Plus 24% HMC	AH Plus 40% HMC	AS Plus 40% HMC	SE	$P > F^2$		
						Forage	HMC	Forage × HMC
Trial 1								
DMI, kg/d	22.8	21.9	24.2	23.5	0.4	0.044	<0.001	0.784
Milk, kg/d	29.6	28.2	31.6	31.8	0.7	0.351	<0.001	0.262
Fat, %	3.84	3.94	3.81	3.88	0.08	0.314	0.555	0.874
Protein, %	3.35	3.29	3.50	3.40	0.03	0.005	<0.001	0.411
Lactose, %	4.73	4.71	4.79	4.79	0.02	0.622	0.007	0.649
SNF, %	8.69	8.70	8.99	8.89	0.06	0.518	<0.001	0.373
Yield, kg/d								
Fat	1.11	1.08	1.17	1.22	0.02	0.668	<0.001	0.091
Protein	0.96	0.90	1.06	1.07	0.02	0.219	<0.001	0.091
Lactose	1.38	1.31	1.46	1.52	0.03	0.865	<0.001	0.062
SNF	2.51	2.40	2.74	2.82	0.05	0.788	<0.001	0.100
Efficiency, milk/DMI	1.32	1.30	1.31	1.37	0.03	0.577	0.350	0.203
	AH	AS	AH Plus 3% FM	AS Plus 3% FM		Forage	FM	Forage × FM
Trial 2								
DMI, kg/d	26.2	24.7	25.8	25.0	0.3	<0.001	0.962	0.367
Milk, kg/d	40.7	39.4	40.9	41.1	0.4	0.112	0.009	0.039
Fat, %	3.25	3.48	3.30	3.36	0.05	0.008	0.513	0.132
Protein, %	3.14	3.10	3.17	3.17	0.02	0.185	0.004	0.382
Lactose, %	4.83	4.84	4.81	4.84	0.01	0.113	0.721	0.630
SNF, %	8.66	8.65	8.69	8.68	0.02	0.578	0.204	0.844
Yield, kg/d								
Fat	1.32	1.34	1.34	1.38	0.02	0.250	0.211	0.953
Protein	1.27	1.20	1.29	1.30	0.02	0.105	<0.001	0.030
Lactose	1.96	1.88	1.97	2.00	0.03	0.318	0.007	0.042
SNF	3.51	3.35	3.56	3.58	0.04	0.148	0.002	0.044
Efficiency, milk, DMI	1.58	1.60	1.59	1.67	0.02	0.009	0.029	0.195

[1]AH = Alfalfa hay, AS = alfalfa silage, HMC = high moisture corn, and FM = fish meal.

[2]Probability of a significant contrast effect.

0.001) in response to HMC. Excretion of PD also was affected ($P \leq 0.051$) by an interaction of forage and HMC; HMC elicited a greater increase for cows fed the AS diets (20 to 25%) than for cows fed the AH diets (3 to 10%). Excretion of PD in milk increased ($P < 0.001$) in response to HMC but was not affected by either forage source or the interaction of forage and HMC. Total excretion of PD (urine plus milk) mainly reflected urinary excretion of PD, increasing ($P < 0.001$) in response to AH and HMC; response to HMC was greater for cows fed the AS diets than for cows fed the AH diets (interaction of forage and HMC; $P = 0.049$). Allantoin accounted for 86.6% of the total excretion of PD, which was intermediate to the 85.5 to 90.6% in previous reports (15, 29, 30).

Secretion of PD in milk accounted for 7.3% of total PD excretion, which was substantially higher than previous reports of 1.6% (15, 29). Mean concentrations of allantoin in milk in the present experiment (1.3 m*M*) more nearly resembled the previous finding of 1.0 m*M* by Vagnoni et al. (29) than that (0.25 m*M*) reported by Giesecke et al. (15). Concentrations of allantoin in plasma followed the general pattern of excretion of allantoin in urine, increasing ($P = 0.006$) in response to increased HMC in the diet and increasing to a greater extent for cows fed the AS diets than for cows fed the AH diets (interaction of forage and HMC, $P = 0.081$).

Ratios of urinary allantoin to urinary creatinine and total PD to creatinine responded similarly to urinary allantoin and total excretion of PD; ratios were higher ($P < 0.001$) for cows fed the AH diets than for cows fed the AS diets, increasing ($P < 0.001$) with greater amounts of HMC and responding more to HMC in AS diets than in AH diets (interaction of forage and HMC, $P \leq 0.021$). Although excretion of creatinine in urine is presumed to be proportional to lean body mass (23) and unaffected by diet, the magnitude of the increase (4%; $P < 0.001$) with increased HMC did not confound interpretation of urinary PD to urinary creatinine ratios.

The bacterial CP to purine ratio (Table 5) was lower for cows fed the AH diets than for cows fed AS diets ($P < 0.001$) and decreased in response to dietary HMC ($P = 0.010$). The ratio also decreased in a linear and quadratic fashion ($P < 0.001$) with respect to time after feeding (Figure 2). Moreover, the interaction ($P = 0.068$) between time after feeding and the amount of HMC occurred because the effect of HMC became smaller with time (Figure 2). Under the assumption that microbial growth rate increased in response to feeding and to increased dietary HMC, these data are consistent with the positive linear relationship between the RNA to protein ratio and the bacterial growth rate that were reported by Bates et al. (3). Estimation of MCP flow from the excretion of PD depends as much upon knowing the CP to purine ratio of ruminal microbes as does the use of purine flow in the digesta. Because of the extensive variation of this ratio in a literature survey [Clark et

TABLE 5. Dietary effects on excretion and concentration of purine derivatives (PD) in plasma and the ratio of CP to purine (grams per millimole) of ruminal bacteria (trial 1).[1]

Item	AH Plus 24% HMC	AS Plus 24% HMC	AH Plus 40% HMC	AS Plus 40% HMC	SE	$P > F$[2] Forage	HMC	Forage × HMC
Urinary excretion, mmol/d								
Creatinine	140	138	144	144	1	0.708	<0.001	0.517
Allantoin	449	380	492	457	8	<0.001	<0.001	0.051
Uric acid	67.3	55.2	69.0	68.8	2.6	0.029	0.008	0.034
Total PD	517	435	561	526	10	<0.001	<0.001	0.027
Milk secretion, mmol/d								
Allantoin	28.0	28.8	33.0	31.3	1.0	0.630	<0.001	0.209
Uric acid	7.73	7.42	9.13	9.79	0.41	0.671	<0.001	0.242
Total PD	35.8	36.3	42.2	41.1	1.2	0.800	<0.001	0.525
Total[3] excretion, mmol/d								
PD	552	473	603	568	11	<0.001	<0.001	0.049
Urine								
Allantoin:creatinine	3.23	2.75	3.43	3.18	0.05	<0.001	<0.001	0.021
Total PD:creatinine	3.71	3.16	3.91	3.66	0.05	<0.001	<0.001	0.009
Plasma concentration								
Allantoin, mg/L	41.6	39.6	43.2	46.4	1.4	0.661	0.006	0.081
Bacterial CP:purine, g/mmol	3.78	4.46	3.56	4.16	0.01	<0.001	0.010	0.553

[1]AH = Alfalfa hay, AS = alfalfa silage, and HMC = high moisture corn.

[2]Probability of a significant contrast effect.

[3]Excretion in urine and secretion in milk.

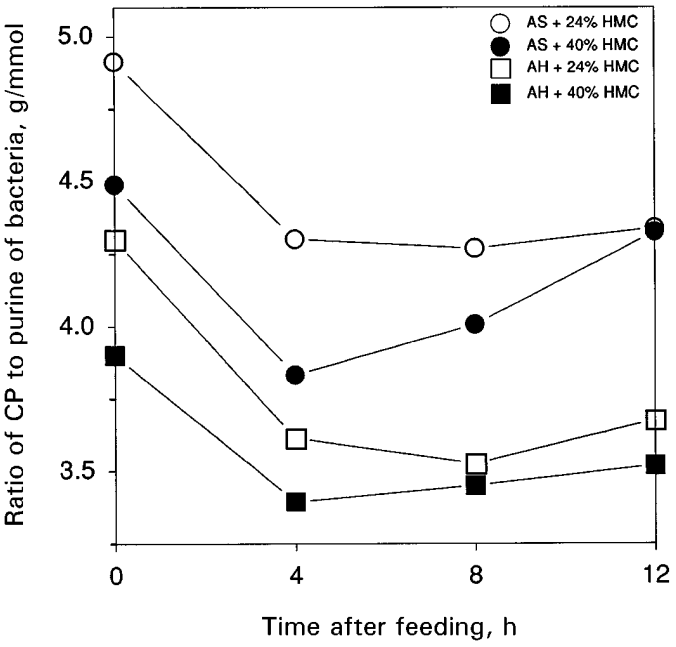

Figure 2. Hourly means from trial 1 for the ratio of CP to purine (grams per millimole) of ruminal bacteria (pooled SE = 0.01). AS = Alfalfa silage, AH = alfalfa hay, and HMC = high moisture corn.

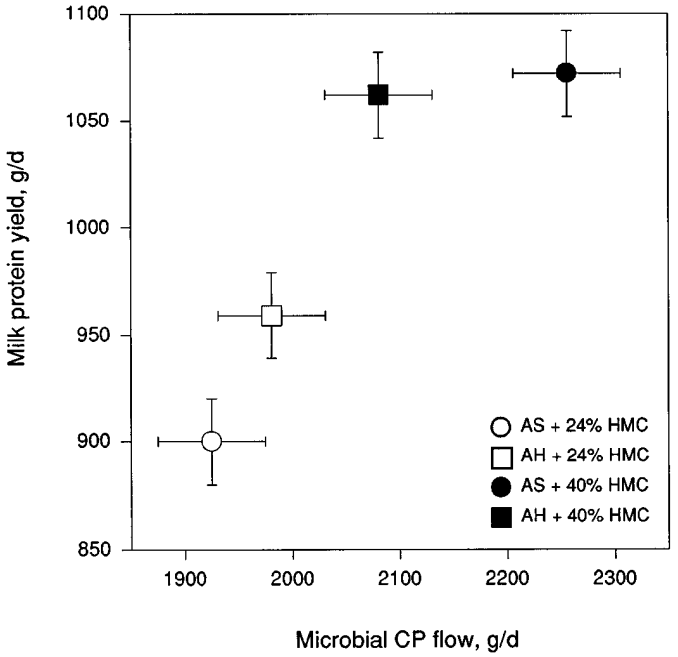

Figure 3. Relationship of milk protein yield and microbial CP flow (trial 1). Bars correspond to pooled standard errors. AS = Alfalfa silage, AH = alfalfa hay, and HMC = high moisture corn.

al. (10) reported a CV of 30% for the CP to purine ratio across studies], quantitative estimation of MCP from the excretion of PD may require determination of microbial CP to purine ratios from samples pooled over time, as was recommended for methods based on purines (7).

Estimated MCP flow (Table 6) increased ($P < 0.001$) in response to dietary HMC; the response to HMC was about three times greater for cows fed the AS diets (337 g/d) than for cows fed the AH diets (100 g/d) (interaction of forage and HMC, $P = 0.022$). Milk protein yield increased linearly as MCP flow increased up to about 1060 g/d, at which point something other than absorbable protein might have limited milk protein secretion (Figure 3). To our knowledge, this study is the first report to estimate MCP from the excretion of PD in dairy cows. Our estimates of MCP yield more nearly resembled those predicted from OM intake (10) than those predicted from NE_L intake (22). However, the equations of both the NRC (22) and Clark et al. (10) considered MCP yield to be strictly limited by energy; dietary N and other factors were not considered to be limiting. Moreover, although those equations demonstrated

TABLE 6. Comparison of intestinal microbial CP flow (grams per day) as estimated by various methods (trial 1).[1]

Method	AH Plus 24% HMC	AS Plus 24% HMC	AH Plus 40% HMC	AS Plus 40% HMC	SE	$P > F$[2] Forage	HMC	Forage × HMC
Trial 1[3]	1981	1925	2081	2262	50	0.222	<0.001	0.022
NRC[4]	2319	2314	2664	2650				
Clark et al. (10)[5]	2020	1945	2135	2077				

[1]AH = Alfalfa hay, AS = alfalfa silage, and HMC = high moisture corn.

[2]Probability of a significant contrast effect.

[3]MCP = (grams of MCP/millimole of purine) × [(millimoles of total PD excreted per day – 103)/0.856] (29).

[4]MCP = 6.25 × (–30.9 + 11.45 × megacalories of NE_L intake per day) (22).

[5]MCP = 6.25 × (21.5 + 14.69 × kilograms of OM intake per day) (10).

that energy intake accounts for the majority of the observed variation in MCP flow ($r^2 \geq 0.62$), they appeared inadequate for detecting important responses to dietary N. The greater response of MCP to HMC for cows fed the AS diets suggested that the extensive ruminal degradation of silage N exceeded the availability of energy for microbial protein synthesis. Although the NRC (22) listed RUP values of 23 and 28% for AS and AH, respectively, Makoni et al. (19) observed an RUP of only 10% for AS. Higher ruminal concentrations of NH_3 and total free AA (Table 3 and Figure 1) in cows fed the AS diets than in cows fed the AH diets were consistent with previous observations (4) and supported the concept of excessive ruminal degradation of silage CP.

Trial 2

Ruminal pH (Table 3) was unaffected ($P \geq 0.436$) by diet; overall mean pH (6.15) was similar to that in trial 1 (6.07). As in trial 1, concentrations of ruminal NH_3 and total free AA were greater ($P \leq 0.039$) for cows fed the AS diets than for cows fed the AH diets, reflecting the high NPN content (Table 2) and extensive ruminal degradability of CP in AS. Consistent with previous observations (4), FM caused a small (1 m*M*; $P = 0.302$) increase in ruminal NH_3 concentration, indicating a minor contribution of FM to the ruminal pool of degradable N. Total VFA ($\overline{X}$ = 162 m*M*) were higher than those for trial 1, likely reflecting the greater DMI in this trial. Although not significant ($P = 0.166$), total VFA were 6% higher in the absence of FM. The molar proportion of acetate and propionate and the ratio of acetate to propionate were unaffected ($P \geq 0.181$) by diet. As in trial 1, molar proportions of butyrate were higher ($P < 0.001$) for cows fed the AS diets than for cows fed the AH diets.

Consistent with results of trial 1 and previous observations (4), DMI was greater ($P < 0.001$) for cows fed the AH diets than for cows fed the AS diets (Table 4); neither FM nor the interaction of forage and FM affected DMI. Previously (4), FM increased DMI by 1 kg/d for cows fed the AS diets but had no effect on DMI of cows fed the AH diets. Digestibility coefficients for dietary NDF and ADF were generally increased by FM and to a greater extent for cows fed the AS diets than for cows fed the AH diets (4), which might have explained the observed responses of DMI to FM. Digestibilities were not measured in the present experiment. Yields of milk, protein, lactose, and SNF all were increased ($P \leq 0.009$) by RUP supplementation as FM; responses to FM ranged from 0 to 2% for cows fed the AH diets and from 5 to 8% for cows fed the AS diets, resulting in interactions ($P \leq 0.044$) between forage source and FM. Responses of milk protein to FM for cows fed the AH diets (20 g/d) and for cows fed the AS diets (100 g/d) were similar to those obtained previously (30 and 100 g/d, respectively) when forage constituted 68% of dietary DM (4). Moreover, these results were consistent with results of trial 1, suggesting that the protein status of cows fed AS is poorer and, thus, more responsive to RUP, than that of cows fed AH. Concentration of milk fat was higher ($P = 0.008$) in response to AS diets than in response to AH diets, and the concentration of milk protein was increased ($P = 0.004$) because of the supplementation of FM.

As was observed earlier (4), efficiency of milk yield (milk/DMI) was greater ($P = 0.009$) for cows fed the AS diets than for cows fed the AH diets. In each case, DMI was lower for cows fed the AS diets than for cows fed the AH diets; previously (4), cows consuming AS appeared to make relatively greater use of tissue reserves to support milk yield (as was evidenced by changes in BW). Production efficiency also was increased ($P = 0.029$) by FM; previously (4), FM had no effect. Changes in the efficiency of milk yield appeared to be mediated by responses in DMI. Responses in milk yield to FM were (4) or were not (trial 2) accompanied by increases in DMI.

CONCLUSIONS

Ruminal NH_3 and total free AA concentrations were higher for cows fed the AS diets than for cows fed the AH diets, suggesting greater ruminal degradation of CP in alfalfa harvested as silage than as hay. The DMI of cows fed AH was greater than that of cows fed AS. Flow of MCP, as estimated from the excretion of PD, and milk protein response to diet were similar. Thus, PD excretion was likely a suitable methodology for estimating MCP flow and milk protein response to MCP yield. Isolation of representative samples of ruminal microbes remains an unresolved problem in estimating MCP flow. Yield of milk protein was increased by supplementation with either ruminally fermentable energy (as HMC) or RUP (as FM). The responses to both HMC and FM were greater for cows fed AS than for cows fed AH, confirming earlier work that showed that the protein status of cows fed AS was poorer than that of cows fed AH.

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